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Evaluation of Antimicrobial Efficacy of ZnO Nanoparticles Against Staphylococcus Aureus and Escherichia Coli

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ABSTRACT
Zinc oxide nanoparticles (ZnO NPs) are widely used antimicrobials whose exact mechanism of action is still being explored. This study investigates the concentration-dependent efficacy of pre-synthesized ZnO NPs of varying sizes against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. The nanoparticles were characterized using standard techniques including X-ray Diffraction, FT-IR Spectroscopy, Thermo-Gravimetric Analysis, and Transmission Electron Microscopy. All bacterial strains were cultured in Mueller Hinton (MH) broth. The minimum inhibitory concentration (MIC) for each set of nanoparticles was determined against the bacterial strains using the standard micro-dilution method. The results confirmed that ZnO NPs exhibit antimicrobial activity against the food borne pathogens *S. aureus* and *E. coli*

KEYWORDS: ZnO nanoparticles, Antimicrobial activity, Staphylococcus Aureus, Escherichia Coli.

1. INTRODUCTION

ZnO, a 3.36 eV wide band-gap semiconductor, is gaining interest in electronics due to its unique properties [1]. Its versatile nanostructures are well-suited for applications in nanoscale optoelectronics [2], piezoelectric nanogenerators [3], and biotechnology [4]. ZnO also exhibits strong resistance to microorganisms [5]. The significant antibacterial activity of ZnO, CaO, and MgO is linked to the surface generation of reactive oxygen species, as shown in conductometric studies [6]. These inorganic oxides provide potent antimicrobial effects at minimal doses while offering essential mineral elements to the human body. Activity is evaluated by monitoring changes in bacterial metabolism within a growth medium. Sawai et al. [7] employed indirect conductometric assays to compare the minimal inhibitory concentration (MIC) of insoluble ceramic powders against various antibiotics. Their research revealed that the antibacterial efficacy of these powders is dependent on particle size, which is controlled by specific processing parameters.

Inorganic antibacterial agents' efficacy often correlates with particle size [8, 9]. Nanocrystalline metal oxides are highly promising due to large surface areas and superior suitability for biological systems [12, 13]. These materials offer advantages over organic agents including enhanced durability, lower toxicity,

greater selectivity, and better heat resistance. TiO_2 and ZnO are well-known semiconductor antimicrobial agents, effective for therapy due to their photocatalytic action under UV light [10, 11]. These agents kill microbes through surface binding followed by cellular internalization. While ZnO's antimicrobial potential is extensively studied, most research uses large particles. Yamamoto [14] linked particle size (0.1–1 μm) directly to efficacy, concluding that smaller ZnO particles exhibited stronger antibacterial activity

2. MATERIAL AND METHOD

Materials

Zinc Nitrate Hydrated (Zn $(NO_3)_2.6H_2O$), Gelatin powder were used.

Bacterial Strains

Bacterial strains of Gram-negative bacteria Escherichia coli and Gram-positive bacteria Staphylococcus aureus were used. All the bacterial strains were grown and maintained in Mueller Hinton (MH) broth. The Minimum inhibitory concentration (MIC) of the different ZnO nanoparticles against the bacterial strains was measured by using standard microdilution method.

Synthesis of ZnO Nanoparticles

ZnO nanopowder was synthesized via a sol-gel technique. The complete procedure of the synthesis of different size ZnO nanoparticles at different calcinations temperatures is already reported by Ajay et al. [15] in their previous study.

3. ANTI-BACTERIAL TEST

Preparation of ZnO Suspension for Anti-bacterial Test

To make ZnO suspension for the antibacterial tests, a preset amount of dry ZnO nanoparticles of different sizes was mixed with distilled water in a glass beaker and is allowed to gently get mix. Once particles were dispersed in water, the beaker was placed in an ultrasonicator. The ultrasonicator was used in order to break down the agglomerates dry ZnO nanoparticles. After 30 min of sonication, the ZnO suspension was produced, which had a concentration of 1 mg/ml. The suspension was then diluted with distilled water to different concentrations for the antibacterial tests. To enhance the stability of the suspension, dispersants PEG-600 was used to produce suspensions of ZnO nanoparticles and the amount of the dispersant was 10% of the amount of zinc oxide nanoparticles. The suspensions prepared were then autoclaved at 121_C for 15 min before using for the anti-bacterial test. Various concentration of ZnO nanoparticle that was for anti-bacterial used are listed below in Table 1.

Table 1: ZnO suspension concentration

Sample		Crystal size	Dispersant	Concentration
A	ZnO@500°C	30 nm	With	1mg/ml
A_d	ZnO@500°C	30 nm	Without	1mg/ml
B	ZnO@600°C	50 nm	With	1mg/ml
B_d	ZnO@600°C	50 nm	Without	1mg/ml
C	ZnO@900°C	150 nm	With	1mg/ml
C_d	ZnO@900°C	150 nm	Without	1mg/ml
D	ZnO@700°C	70 nm	With	1mg/ml
D_d	ZnO@700°C	70 nm	Without	1mg/ml

Anti-bacterial Test using Microdilution Method

Anti–bacterial test were performed using micro-dilution method. The Serial dilutions of the ZnO particle suspension were made from 10001µg/ml to 1.5 µg/ml in the microdilution wells as labelled 1-10 respectively. The MH broth (100 µl) were mixed with inoculums of different bacterial strains (100 µl of 10^5 colony forming units per ml) from log phase of bacteria and incubated with shaking for 24h at 37^0C. The shaking is done in order to prevent the settling down of the ZnO nanoparticles .MIC was taken as the lowest concentration at which no visible growth of bacteria was observed.

Inoculum Preparation

Asingle colony was selected from a plate and used to inoculate 10mL of Mueller Hinton (MH) broth media. This primary culture was incubated in a shaking incubator at 37°C and 200rpm for 16-18 hours. Following this initial incubation, 100µL of the primary culture was transferred into a fresh 10mL volume of MH broth media. This secondary culture was then incubated under the same conditions (37°C at 200rpm) until its optical density at 600nm reached 1.0, as measured by a spectrophotometer. The resulting culture was subsequently diluted to achieve a final concentration of 10^5 CFU per mL. Finally, 100µL of this diluted culture was dispensed into each well of a 96-well microtiter plate

Broth Microdilution Assay:

One hundred microliters (100 µL) of sterile Mueller-Hinton Broth (MHB) was added to wells 2 through 11 of a microtiter plate, while well 12 received 200 µL of unsterile MHB to serve as the negative control. Well 1 was initially filled with 200 µL of a ZnO suspension. A serial dilution was then performed by transferring 100 µL of the ZnO suspension sequentially from well 1 to well 10, discarding 100 µL from well 10 afterward. Subsequently, 100 µL of a bacterial culture (10^4 CFU) was introduced into wells 1 through 11. Well 11, containing no ZnO suspension, functioned as the positive control. The covered plates were incubated at 37°C for 16–18 hours. Following incubation, the minimum inhibitory concentration (MIC) values (in mg/L) were determined by observing which wells showed a lack of turbidity or bacterial growth.

4. RESULTS AND DISCUSSIONS

The characterization results of FTIR spectroscopy, X-Ray diffraction pattern, TEM spectroscopy and Thermo-gravimetric analysis of the same ZNO nanoparticles were already discussed and reported by Ajay et al. (2025) in the previous study [15].

ZnO nanoparticles showed a strong antimicrobial activity for Escherichia coli and Staphylococcus aureus. In case of Staphylococcus aureus complete inhibtion for the bacterial strain obtained at the concentration of 1000 µg/ml. Althought at concentration of 500 µg/ml showed partial inhibition in all the different size nanoparticles and at concentration of 250 µg/ml showed partial inhibition in ZnO suspensions C, D, D_d. Antibacterial activity very strongly dependent on the size of nanoparticle . As shown below in Figure 1. ZnO suspensions showed any difference strong antibacterial activity in both the cases, with or without using dispersants. Size dependent Antibacterial activity of ZnO nanoparticle is shown in the Table 2 below.

Table 2. Microtitre dilution results for Staphylococcus aureus

Well	1	2	3	4	5	6	7	8	9	10	11	12
µg/ml	1000	500	250	125	62	31	15	7	3.9	1.95	No particles	No particles & inoculums
Zno@500°C (A)	-	+	++	++	++	++	++	++	++	++	++	-
Zno@500°C (A_d)	-	+	++	++	++	++	++	++	++	++	++	-
Zno@600°C (B)	-	+	++	++	++	++	++	++	++	++	++	-
Zno@600°C (B_d)	-	+	++	++	++	++	++	++	++	++	++	-
Zno@900°C (C)	-	+	++	++	++	++	++	++	++	++	++	-
Zno@900°C (C_d)	-	+	+	++	++	++	++	++	++	++	++	-
Zno@700°C (D)	-	+	+	++	++	++	++	++	++	++	++	-
Zno@700°C (D_d)	-	+	+	++	++	++	++	++	++	++	++	-

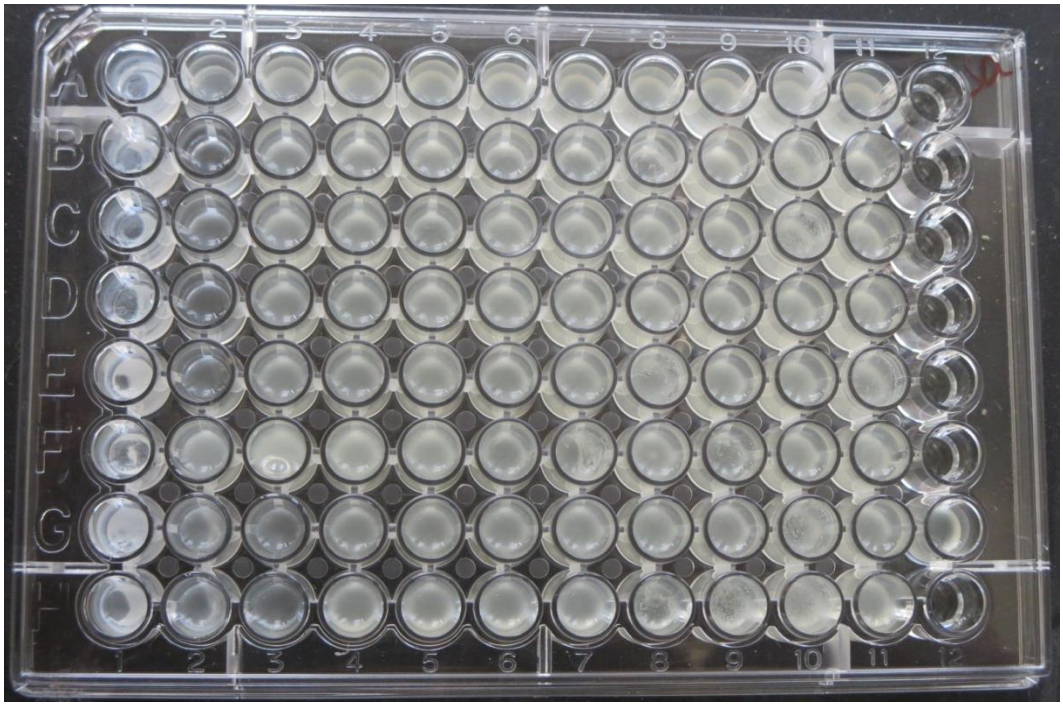

Figure 1. Microtitre dilution results forStaphylococcus aureus

In case of *Escherichia coli* complete inhibtion for the bacterial strain obtained at the concentration of 500 µg/ml. Althought at concentration of 250 µg/ml showed partial inhibition in all the different sized nanoparticles suspension other than ZnO suspension C, C_d. Antibacterial activity very strongly dependent on the size of nanoparticle. Figure 2 shows as the size of nanoparticle is decreased anti bacterial activity against E.coli. A increased ZnO suspensions showed any difference strong antibacterial activity in both the cases, with or without using dispersants. Size dependent Antibacterial activity of ZnO nanoparticle is shown in the Table 3 below

Table 3. Microtitre dilution results for E.coli

Well	1	2	3	4	5	6	7	8	9	10	11	12
µg/ml	1000	500	250	125	62	31	15	7	3.9	1.95	No particles	No particles& inoculums
Zno@500°C (A)	-	-	+	++	++	++	++	++	++	++	++	-
Zno@500°C (A_d)	-	-	+	++	++	++	++	++	++	++	++	-
Zno@600°C (B)	-	-	+	++	++	++	++	++	++	++	++	-
Zno@600°C (B_d)	-	-	+	++	++	++	++	++	++	++	++	-
Zno@900°C (C)	-	-	+	++	++	++	++	++	++	++	++	-
Zno@900°C (C_d)	-	-	++	++	++	++	++	++	++	++	++	-
Zno@700°C (D)	-	-	+	++	++	++	++	++	++	++	++	-
Zno@700°C (D_d)	-	-	+	++	++	++	++	++	++	++	++	-

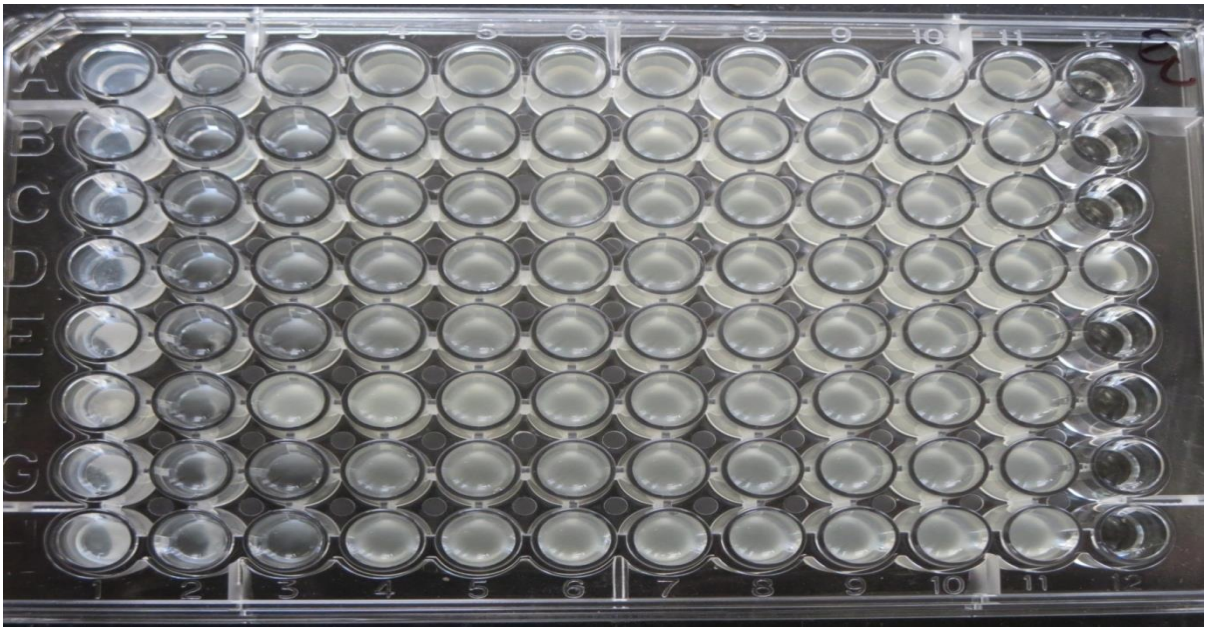

Figure 2. Microtitre dilution results for E.coli.

5. CONCLUSION

ZnO NPs act as an impressive antibacterial agent against food borne pathogen Staphylococcus aureus and E. coli. The minimium inhibition concentrations were found to be 1000µg/ml and 500µg/ml respectively.

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